1: J Biol Chem 1990 Sep 15;265 (26):15813-7

Cloning of ubiquitin activating enzyme from wheat and expression of a functional protein in Escherichia coli.

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The initial step in the conjugation of ubiquitin to substrate proteins involves the activation of ubiquitin by ubiquitin activating enzyme, E1. Previously, we purified and characterized multiple species of E1 from wheat germ. We now describe the isolation and characterization of a cDNA clone encoding E1 from wheat. This clone (UBA1) was isolated from a cDNA expression library with anti-wheat E1 antibodies. It contained an open reading frame coding for 1051 amino acids and directed the synthesis of a protein that comigrated with a wheat germ E1 of 117 kDa. UBA1 was confirmed as encoding E1 by (i) comparison of the peptide map of the protein product of UBA1 synthesized in Escherichia coli with that of purified E1 from wheat, and (ii) amino acid sequence identity of peptides generated from purified E1 with regions of the derived amino acid sequence of UBA1. The isolation of two additional cDNAs closely related to UBA1 indicated that E1 was encoded by a small gene family in wheat. Nonetheless, a single poly(A+) mRNA size class of 4 kilobases hybridized with UBA1. When expressed in E. coli, the product of UBA1 catalyzed the formation of a thiol ester linkage between ubiquitin and an ubiquitin carrier protein. The ability of E. coli containing UBA1 to synthesize an active protein will allow us to identify domains important for E1 function using in vitro mutagenesis.

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1: J Biol Chem 1999 May 21;274(21):14823-30

Identification of the ubiquitin carrier proteins, E2s, involved in signal-induced conjugation and subsequent degradation of IkappaBalpha.

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The last step in the activation of the transcription factor NF-kappaB is signal-induced, ubiquitin- and proteasome-mediated degradation of the inhibitor IkappaBalpha. Although most of the components involved in the activation and degradation pathways have been identified, the ubiquitin carrier proteins (E2) have remained elusive. Here we show that the two highly homologous members of the UBCH5 family, UBCH5b and UBCH5c, and CDC34/UBC3, the mammalian homolog of yeast Cdc34/Ubc3, are the E2 enzymes involved in the process. The conjugation reaction they catalyze in vitro is specific, as they do not recognize the S32A,S36A mutant species of IkappaBalpha that cannot be phosphorylated and conjugated following an extracellular signal. Furthermore, the reaction is specifically inhibited by a doubly phosphorylated peptide that spans the ubiquitin ligase recognition domain of the inhibitor. Cys-to-Ala mutant species of the enzymes that cannot bind ubiquitin inhibit tumor necrosis factor alpha-induced degradation of the inhibitor in vivo. Not surprisingly, they have a similar effect in a cell-free system as well. Although it is clear that the E2 enzymes are not entirely specific to IkappaBalpha, they are also not involved in the conjugation and degradation of the bulk of cellular proteins, thus exhibiting some degree of specificity that is mediated probably via their association with a defined subset of ubiquitin-protein ligases. The mechanisms that underlie the involvement of two different E2 species in IkappaBalpha conjugation are not clear at present. It is possible that different conjugating machineries operate under different physiological conditions or in different cells.

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Association with cullin partners protects ROC proteins from proteasome-dependent degradation.

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Cullin 1/CDC53 represents a multigene family and has been linked to the ubiquitin-mediated proteolysis of several different proteins. We recently identified two closely related RING finger proteins, ROC1 and ROC2, that share considerable sequence similarity to an APC subunit, APC11, and demonstrated ROC1 as an essential subunit of CUL1 and CDC53 ubiquitin ligases. We report here that the expression of ROC1, ROC2 and APC11 genes are induced by mitogens and remain constant during the cell cycle. Unlike other subunits of SCF and APC E3 ligases, ectopically expressed ROC family proteins are degraded by a proteasome-inhibitor sensitive pathway and are stabilized by associating with cullins. Mutations at the conserved Phe79 and His80 residues in the RING finger of ROC1 diminish its binding with cullins, resulting in a loss of cullin protection and ubiquitin ligase activity. These results suggest a potential mechanism for regulating the activity of ROC-cullin ligases through complex assembly and ROC/APC11 subunit ubiquitination.

PMID: 10597284 [PubMed - indexed for MEDLINE]

1: J Biol Chem 2000 Sep 1;275(35):26661-4 Related Articles, NEW Books, LinkOut

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The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7.

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The inhibitor of apoptosis, cIAP2, contains a putative Ring finger motif at the C terminus. Using in vitro ubiquitination assays, we found that the Ring finger of cIAP2 alone possesses intrinsic ubiquitin ligase activity and promotes substrate-independent ubiquitination. It also promotes ubiquitination of caspases 3 and 7 but not caspase-1. The Ring fingers of c-Cbl and Apc11 failed to promote caspase-7 ubiquitination, suggesting that the Ring finger of cIAP2 itself is involved in substrate recognition.

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